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How do leaf and ecosystem measures of water-use efficiency compare?

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Abstract

The terrestrial carbon and water cycles are intimately linked: the carbon cycle is driven by photosynthesis, while the water balance is dominated by transpiration, and both fluxes are controlled by plant stomatal conductance. The ratio between these fluxes, the plant water use efficiency (WUE), is a useful indicator of vegetation function. WUE can be estimated using several techniques, including leaf gas exchange, stable isotope discrimination, and eddy covariance. Here we compare global compilations of data for each of these three techniques. We show that patterns of variation in WUE across plant functional types are not consistent among the three datasets. Key discrepancies include: (1) Leaf-scale data indicate differences between needle-leaf and broadleaf forests, but ecosystem-scale data do not; (2) leaf-scale data indicate differences between C3 and C4 species, whereas at ecosystem scale there is a difference between C3 and C4 crops but not grasslands; and (3) isotope-based estimates of WUE are higher than estimates based on gas exchange for most PFTs. Our study quantifies the uncertainty associated with different methods of measuring WUE, indicates potential for bias when using WUE measures to parameterise or validate models, and indicates key research directions needed to reconcile alternative measures of WUE.

Keywords: stomatal conductance, water use efficiency, plant functional type, stable isotopes, leaf gas exchange, eddy covariance

Introduction

One of the fundamental trade-offs governing plant growth is the exchange of water for carbon: land plants must open their stomata to take up carbon dioxide in order to grow, but at the same time water vapour is lost via transpiration, with the concomitant risk of desiccation (Cowan & Farquhar 1977). This trade-off can be characterised by the plant's water-use efficiency (WUE), defined as the amount of carbon taken up per unit water used (Sinclair et al. 1984). Combining as it does the key processes of photosynthesis and transpiration, WUE is a widely-used parameter indicating vegetation performance.

Water-use efficiency can be estimated using several methods that operate at different temporal and spatial scales. Community research efforts have led to the compilation of global datasets based on each of these methods. These datasets are increasingly being utilised to constrain and evaluate global vegetation models (e.g. Groenendijk et al. 2011, Saurer et al. 2014, Kala et al. 2015, Dekker et al. 2016). However, there has to date been little comparison across methods. It is often assumed that values obtained at one scale should be relatable to values obtained at other scales, but this assumption has not been explicitly tested across ecosystems. Our goal in this paper is to compare three independent global datasets of WUE, obtained using leaf gas exchange, stable isotope, and eddy covariance techniques, and investigate whether global patterns obtained using these different techniques are consistent with our current understanding of scaling. Specifically, we focus on patterns of variation across plant functional types (PFTs), which are used to represent vegetation in global vegetation models, and ask whether the three datasets indicate consistent differences among PFTs.

WUE is known to vary with atmospheric vapour pressure deficit (VPD) (Monteith 1986). To compare across datasets, a metric of WUE is required that accounts for this variation. One commonly-used metric is the intrinsic WUE (iWUE), defined as photosynthetic C uptake divided by stomatal conductance to water vapour (A/g_s). Another related metric is the ratio of intercellular to atmospheric CO₂ (C_i/C_a ratio). However, both iWUE and the C_i/C_a ratio also vary with VPD, meaning that values obtained under different VPD

conditions cannot be directly compared. In this work, we account for variation in VPD conditions by using the parameter, g_1 , of a recent model of stomatal conductance (g_s mol $m^{-2} s^{-1}$), derived from the theory of optimal stomatal behaviour (Medlyn et al. 2011):

$$g_s = 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_s} \quad (1)$$

where A is the net assimilation rate ($\mu mol m^{-2} s^{-1}$), C_s ($\mu mol mol^{-1}$) and D (kPa) are the CO_2 concentration and the vapour pressure deficit at the leaf surface, respectively. The model parameter g_1 ($kPa^{0.5}$) represents normalised plant water use efficiency. The model parameter g_1 is inversely related to iWUE but accounts for VPD, by assuming a \sqrt{D} dependence of the C_i/C_a ratio, as found for leaf gas exchange (Medlyn et al. 2011) and eddy covariance data (Zhou et al. 2015). ~~and can thus be directly compared across datasets.~~ This parameter also corrects for increases in WUE driven by changes in C_a . If the ratio C_i/C_a is constant with increasing C_a , then g_1 is also constant (Medlyn et al. 2011). Assuming that these relationships accurately account for environmental effects on WUE, the parameter g_1 is then a measure of WUE that can ~~and can thus be directly compared across datasets.~~

We apply this model to three major global data compilations. Lin et al. (2015) compiled a global database of leaf gas exchange measurements, including photosynthetic rate and stomatal conductance, and used these data to estimate instantaneous values of g_1 . Lin et al. (2015) found systematic differences in g_1 among PFTs, with high values of g_1 (and thus low iWUE) in crops, C3 grasses and deciduous angiosperm trees, and low values in C4 grasses and gymnosperms. Leaf-level gas exchange data such as these are commonly used to parameterise stomatal behaviour in vegetation models (e.g. Bonan et al. 2014). The differences among PFTs observed by Lin et al. (2015) have important consequences for modelled vegetation function at large scales, including changes in predicted surface cooling and consequent heatwave development (Kala et al. 2015, 2016).

Stable isotope methods can be applied to plant tissue to estimate $iWUE$ and g_1 values over monthly to annual time scales (Farquhar et al. 1989, Cernusak et al. 2013). Long-term stable isotope records from tree rings are widely used to constrain model predictions of WUE at large spatial and temporal scales (e.g. Frank et al. 2015, Saurer et al. 2015, Dekker et al. 2016). A compilation of leaf ^{13}C discrimination measurements indicated differences in stomatal behaviour among PFTs (Diefendorf et al. 2010). Here, we estimated g_1 values from a global database of nearly 4,000 measurements of bulk leaf ^{13}C discrimination ($\Delta^{13}C$), taken from 594 sites spread across all seven continents (Cornwell et al, in review). We predicted that values of g_1 estimated from this dataset would show similar rankings across PFTs as the leaf gas exchange data set, but that values would be lower, due to mesophyll resistance to CO_2 diffusion (Seibt et al. 2008).

At larger spatial scales, eddy flux measurements can be used to estimate whole-ecosystem gross primary productivity (GPP) and evapotranspiration (ET), and their ratio GPP/ET, which is the whole-ecosystem water-use efficiency (Law et al. 2002, Beer et al. 2009, Keenan et al. 2013). These data are also being widely applied to constrain and evaluate vegetation models (e.g. Groenendijk et al. 2011, Bonan et al. 2012, Haverd et al. 2013). We predicted that g_1 values estimated from these data would show similar rankings across PFTs as the leaf gas exchange and stable isotope datasets, but that estimated values of g_1 would be higher due to the contribution of non-transpiratory water vapour fluxes to evapotranspiration (i.e. free evaporation from soil and canopy).

Methods

Datasets

We synthesised three independent datasets to estimate values of g_1 . All datasets, and our analysis code, are available online; web addresses are given below under “data deposition statement”. Leaf gas exchange data were taken from Lin et al. (2015), who collated measurements under ambient field conditions from 286 species, covering 56 sites across the globe. The majority of these data are measurements on upper-canopy leaves during

the growing season. Isotope data came from a global database of leaf carbon isotopes measurements from natural and semi-natural habitats, across 3985 species-sites combinations (Cornwell et al. in review). Flux measurements were taken from the global collection of eddy flux measurements that comprise the FLUXNET “La Thuile” Free and Fair dataset (<http://www.fluxdata.org>). This dataset contains gap-filled, half-hourly measurements of carbon dioxide, water vapour and energy fluxes; following filtering (see below) we were able to use data from 120 sites. The global distribution of the three datasets is shown in Figure 1.

Estimating g_l

g_l was estimated from leaf gas exchange data using non-linear regression to fit the unified stomatal optimisation model (Medlyn et al. 2011, eqn 1) to g_s measurements for each species. Here we followed the methods of Lin et al. (2015). All model fits were done using the “minimize” function of the python “lmfit” library, using the Levenberg-Marquardt method (Newville et al. 2014).

Cornwell et al. (in review) estimated carbon isotope discrimination (Δ) values from bulk leaf $\delta^{13}\text{C}$ and estimates of source air $\delta^{13}\text{C}$ composition. From these data, we estimated the ratio of the intercellular to ambient carbon dioxide concentration (C_i/C_a) following Farquhar et al. (1989) for C_3 species:

$$\frac{C_i}{C_a} = \frac{\Delta - a}{b - a} \quad (2)$$

where a represents the fractionation caused by gaseous diffusion (4.4‰) and b is the effective fractionation caused by carboxylating enzymes (assumed to be 27‰) (Cernusak et al. 2013). Note that we were unable to utilise values for C_4 vegetation from this dataset. For C_4 plants, the relationship between C_i/C_a and $\Delta^{13}\text{C}$ depends on bundle sheath leakiness, ϕ (Henderson et al. 1998; Cernusak et al. 2013). Adopting a value for ϕ of 0.21

for C4 vegetation, as suggested by Henderson et al. (1998), yielded unrealistic estimates of $C_i/C_a < 0$ for more than half (79/140) of the dataset.

Values of g_1 for C3 species were estimated following Medlyn et al. (2011):

$$g_1 = \frac{\left(\frac{C_i}{C_a} \sqrt{D}\right)}{\left(1 - \frac{C_i}{C_a}\right)} \quad (3)$$

Mean daytime growing season VPD was estimated from monthly mean and maximum temperature and relative humidity data obtained from the Climatic Research Unit (CRU 1.0) 0.5-degree gridded monthly climatology (New et al. 2002). Growing season was defined as the time period during which the daytime mean temperature is above zero. All values were estimated on a monthly basis then linearly interpolated to a daily basis. Daily VPD estimates could then be averaged over the growing season.

Values of g_1 were estimated from FLUXNET data as follows. First, canopy stomatal conductance (G_s) was estimated from LE flux ($\text{J m}^{-2} \text{s}^{-1}$) as

$$G_s = \frac{LE/\lambda}{D/P} \quad (4)$$

where λ is the latent heat of water vapour (J mol^{-1}), D (Pa) is the vapour pressure deficit and P is the atmospheric pressure (Pa). Pressure was estimated using the hypsometric equation based on site elevation data. Where site elevation information was missing, values were gap-filled using the 30-arc seconds (~ 1 km) global digital elevation model GTOPO30 data from the United States Geological Survey (USGS). Values of g_1 were then estimated by fitting eqn (1) to data, taking G_s for g_s and gross primary productivity (GPP) for A .

FLUXNET data were screened as follows: (i) data flagged as “good”; (ii) data from the three most productive months, in terms of flux-derived GPP (to account for the different timing of summer in the Northern and Southern hemispheres); (iii) daylight hours

between 9 am and 3 pm; (iv) time slices with precipitation, as well as the subsequent 48 half-hour time slices, were excluded (to minimise contributions from soil/wet canopy evaporation); (v) time slices with missing CO₂ data were gap-filled with the global annual mean from averaged marine surface (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>). If the entire year's data were missing, or if the annual mean departed from the global mean by $\pm 15\%$, data were replaced with the global mean. This screening check was used to address possible errors in locally recorded CO₂ concentrations in 14 site-year combinations, which showed drops against a global trend of increasing CO₂ concentrations (1995–2004: 1.87 ppm yr⁻¹). In addition, fitted g_1 values with an $R^2 < 0.2$ were excluded, as were fitted g_1 values that were $\pm 50\%$ from the site average.

We used equation (4) to estimate canopy conductance as this approach is taken in a number of other studies (e.g. Beer et al. 2009, Keenan et al. 2013) and the equation can be applied to all Fluxnet datasets. However, the use of equation (4) to estimate canopy conductance is a simplification because it assumes that the vegetation is fully coupled to the surrounding atmosphere, and therefore that water vapour exchange is directly proportional to stomatal conductance. There is also an aerodynamic resistance to gas exchange, resulting in a partial decoupling of canopy-atmospheric gas exchange, particularly in short-statured vegetation (Jarvis & McNaughton 1986). To estimate values of g_1 accounting for aerodynamic resistance, G_s was estimated by inverting the Penman-Monteith equation from measured LE flux:

$$G_s = \frac{G_a \gamma \lambda E}{s(R_n - G) - (s + \gamma)\lambda E + G_a M_a c_p D} \quad (5)$$

where G_a (mol m⁻² s⁻¹) is the canopy aerodynamic conductance, λ is the latent heat of water vapour (J mol⁻¹), E (mol m⁻² s⁻¹) is the canopy transpiration, γ is the psychrometric constant (Pa K⁻¹), s is the slope of the saturation vapour pressure curve at air temperature (Pa K⁻¹), R_n (W m⁻²) is the net radiation, D (Pa) is the vapour pressure deficit, G (W m⁻²) is the soil heat flux, M_a (kg mol⁻¹) is molar mass of air, c_p is the heat capacity of air (J kg⁻¹ K⁻¹). At sites where values of G were not available, G was set to zero. G_a was calculated

as $P / (R_{\text{gas}} T_k) / (u/u^* + 6.2u^{*-2/3})$ where u^* (m s^{-1}) is friction velocity and u (m s^{-1}) is wind speed (Thom et al. 1972). P is atmospheric pressure (Pa), R_{gas} is the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$), T_k is the air temperature in Kelvin, and the term $P / (R_{\text{gas}} T_k)$ converts from units of m s^{-1} to $\text{mol m}^{-2} \text{s}^{-1}$. Equation (5) was applied to all datasets where R_n and u^* were available. Inspection of equation (5) shows that, under most conditions, incorporating a finite G_a value will lead to a lower estimate of G_c than would be obtained with infinite G_a .

Ancillary data

The isotope dataset does not contain information on PFTs; these were determined from species information online. If we were unable to assign a PFT, data were excluded from further analysis. For Fluxnet data, the PFTs WSA (woody savannah) and SAV (savannah) were combined into SAV, and PFTs OSH (open shrublands) and CSH (closed shrublands) were combined into SHB. PFT MF (mixed forest) was omitted. Data screening led to a loss of 12% from the isotope dataset and ~35% from the FLUXNET dataset.

Estimates of the relative fraction of C4 present at each FLUXNET site were derived from the closest matching 0.5-degree pixel in the North American Carbon Program (NACP) Global C3 and C4 SYNergetic land cover MAP (SYNMAP) (Jung et al. 2006).

Peak LAI for FLUXNET sites was obtained from the site-level ancillary data when available in the supporting documents contributed to the La Thuile Synthesis Collection (see www.fluxdata.org).

Statistics

We tested for statistical differences among methods by applying one-way ANOVA to log-transformed values of $g_{1\text{-leaf}}$, $g_{1\text{-isotope}}$ and site-averaged $g_{1\text{-flux}}$ for each PFT. For each method, we used a mixed-model approach to test for differences among PFTs, taking site as a random factor. Similarly, a mixed-model approach was used to test for statistical

272 differences among PFTs for a given method. Differences among methods and among
273 PFTs were identified using Tukey's Honest Significant Difference.
274
275

Results

Values of g_1 estimated using the three alternative methods differed significantly within most PFTs (Figure 2). In addition, the variation in g_1 across PFTs was not consistent among the three methods (Table 1).

Forest PFTs

Among the four forest PFTs, median values of g_1 derived from leaf gas exchange ($g_{1\text{-leaf}}$) were lowest in evergreen needleleaf forest (ENF), intermediate in evergreen broadleaf forest (EBF) and highest in deciduous broadleaf forest (DBF) and tropical rainforest (TRF). Isotope-derived values of g_1 ($g_{1\text{-isotope}}$) mostly had similar variation across forest types as $g_{1\text{-leaf}}$ values: they were lowest in ENF, intermediate in EBF and DBF, but were significantly larger in TRF. In clear contrast to other two datasets, there were no significant differences among forest types for values of g_1 derived from flux data ($g_{1\text{-flux}}$). Values of $g_{1\text{-flux}}$ for ENF and EBF were higher than those of the other datasets.

Values of $g_{1\text{-isotope}}$ were generally lower than values of $g_{1\text{-leaf}}$ for a given PFT, with the exception of TRF (Figure 2). The largest difference between $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ was observed for DBF species, whereas there was no significant difference in mean values for EBF and TRF species. For the TRF PFT, $g_{1\text{-isotope}}$ values were often unrealistically high; inferred values of $C_i/C_a > 0.95$ resulted in values of $g_{1\text{-isotope}} > 20 \text{ kPa}^{0.5}$. Such high values were not limited to one dataset, but were observed in a number of TRF datasets.

Non-forest PFTs

Among the non-forest PFTs, $g_{1\text{-leaf}}$ values were significantly higher in C3 grasses (C3G) than C4 grasses (C4G), intermediate in shrubs (SHB), and rather variable in savannah (SAV) trees. The variability of $g_{1\text{-leaf}}$ in SAV is likely related to the high seasonality in these systems: this instantaneous measure of WUE can vary considerably between wet and dry seasons. Note that the comparison among methods for the SAV PFT is somewhat biased because eddy covariance data are from the whole ecosystem and thus include both trees and understorey, whereas leaf gas exchange for this PFT is from trees only while

isotope data are principally from trees and shrubs. As with forest PFTs, values of $g_{1\text{-isotope}}$ for non-forest PFTs were on average lower than values of $g_{1\text{-leaf}}$, but the rankings of PFTs differed: C₃ grasses had lower $g_{1\text{-isotope}}$ values than SAV or SHB, an unexpected result. We were unable to estimate values of $g_{1\text{-isotope}}$ for C₄ species (see Methods) although $\Delta^{13}\text{C}$ values clearly differed between C₃ and C₄ vegetation (Cornwell et al. in review).

Photosynthetic pathway had a significant effect on $g_{1\text{-flux}}$ values for crop vegetation: $g_{1\text{-flux}}$ was significantly lower in C₄ crops (C₄C) than C₃ crops (C₃C). Values of $g_{1\text{-flux}}$ were high for grasslands (C₃G), similar to $g_{1\text{-leaf}}$ values and much higher than $g_{1\text{-isotope}}$ values. We did not find evidence that the presence of C₄ grasses reduced $g_{1\text{-flux}}$ in grasslands (Figure 3); grassland $g_{1\text{-flux}}$ values were not correlated with estimated C₄ fraction.

Comparison of forest and non-forest PFTs

Apart from C₄C, median values of $g_{1\text{-flux}}$ were somewhat higher for non-forest than forest vegetation, and were particularly high for SHB. It is possible that the contribution of soil evaporative flux to total evapotranspiration is higher in these more open systems, resulting in larger $g_{1\text{-flux}}$ values. This conclusion is supported by an examination of the influence of leaf area index (LAI) on $g_{1\text{-flux}}$ for forest and non-forest vegetation, for sites where LAI estimates were available (Figure 4). At lower LAI (up to 3 m² m⁻²), values of $g_{1\text{-flux}}$ were more variable for non-forest than forest sites, with several non-forest sites showing values of $g_{1\text{-flux}}$ greater than 8 kPa^{0.5}, providing some support for the inference that soil evaporative fluxes play a larger role in non-forest ecosystems.

Exploration of inconsistent patterns among datasets

The lack of difference among $g_{1\text{-flux}}$ values for forest PFTs was unexpected. The consistent evidence from $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ values suggests that leaf-scale g_1 is low for ENF. We had anticipated that this difference would scale to canopy behaviour, yet there is no evidence that $g_{1\text{-flux}}$ values were lower for this PFT. It is possible that sampling biases lead to different results for the three methodologies. To investigate this possibility, we first compared the latitudinal distributions of the three datasets, using latitude as an indicator of climatic conditions (Figure 5). Clear differences in sampling coverage with

latitude can be seen. However, Figure 5 demonstrates that irrespective of latitude, values of $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ are lower in ENF than in DBF, whereas values of $g_{1\text{-flux}}$ are similar between ENF and DBF.

To further rule out sampling bias, we also compared half-hourly leaf gas exchange data and eddy flux data for eight sites where both kinds of data were available (Figure 6). This direct comparison shows that $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ values were in a similar range for DBF and TRF forest types but that $g_{1\text{-leaf}}$ was lower than $g_{1\text{-flux}}$ for EBF and ENF forest types, further confirming that the discrepancy between $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ is not simply a result of sampling bias.

We tested whether decoupling of canopy-atmosphere gas exchange could explain the discrepancy between the cross-PFT patterns in $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ values. We estimated canopy stomatal conductance from eddy flux data using the Penman-Monteith (PM) equation (equation 5), which incorporates an aerodynamic resistance term. Applying the PM equation results in a large reduction in estimated values of $g_{1\text{-flux}}$ for all PFTs (Figure 7). For PFTs where $g_{1\text{-flux}}$ previously exceeded $g_{1\text{-leaf}}$, the values become comparable (e.g. ENF). However, for PFTs where $g_{1\text{-flux}}$ was previously comparable with $g_{1\text{-leaf}}$, the values become significantly lower (e.g. DBF, C3G). Thus, consideration of decoupling does not resolve the inconsistency in cross-PFT patterns between $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$.

Discussion

Our comparison of g_1 values across three global datasets provides a number of new insights into patterns of water use efficiency across scales, and highlights some important inconsistencies in the datasets. The parameter g_1 is inversely related to water-use efficiency, such that plants with high WUE have low g_1 and vice-versa. We had predicted that g_1 values would vary consistently across PFTs in all three datasets, but our results did not support this prediction, as there were significantly different patterns across PFTs in each dataset. We also predicted that g_1 values would vary across methods, with the lowest values obtained from isotope data, and the highest values obtained from flux data. The

first part of this prediction was largely supported, with lower $g_{1\text{-isotope}}$ than $g_{1\text{-leaf}}$ for most PFTs, but the second part of the prediction was not, as $g_{1\text{-flux}}$ values were not in general higher than $g_{1\text{-leaf}}$, particularly when decoupling between the canopy and atmosphere was taken into account.

Cross-PFT patterns compared among datasets

For forest vegetation, there was an important discrepancy in cross-PFT patterns between leaf and ecosystem-scale estimates of g_1 . At leaf scale, a difference between needle-leaf (ENF) and deciduous broad-leaf (DBF) forests is seen in both leaf gas exchange and stable isotope data, as has also been found in previous studies (e.g. Lloyd & Farquhar 1994, Diefendorf et al. 2010). Our current understanding of scaling between leaves and ecosystems suggests that a similar difference between these PFTs should be seen in g_1 estimated from eddy covariance data. Intriguingly, however, no such difference was observed; values of $g_{1\text{-flux}}$ were similar for all forest PFTs (Figures 2, 6). This inconsistency between datasets has important consequences for our ability to model water use efficiency at larger scales, since it implies that models parameterised with leaf gas exchange or stable isotope data will not agree with flux data, or with models parameterised using flux data.

Consideration of decoupling between stomata and atmosphere (*sensu* Jarvis & McNaughton 1986) did not help to explain this discrepancy (Figure 7). We found that there was no difference in $g_{1\text{-flux}}$ among forest types irrespective of whether the estimation of $g_{1\text{-flux}}$ incorporated a decoupling factor. We found that median $g_{1\text{-flux}}$ approached median $g_{1\text{-leaf}}$ for needle-leaf forests when decoupling was considered, and for broadleaf forests when it was not. This observation is supported by previous studies of scaling on single forests: a study on water-use efficiency in Scots pine found congruence between leaf and canopy water-use efficiency using a scaling approach incorporating decoupling (Launiainen et al. 2011) whereas studies in broadleaf forests find congruence using approaches that do not consider decoupling (Linderson et al. 2012, Barton et al. 2012). However, it is generally thought that decoupling should be smallest in needleleaf canopies (Jarvis & McNaughton 1986). This discrepancy clearly requires further

investigation. [Refining estimates of canopy stomatal and non-stomatal conductances from eddy flux data is one potential way forwards \(e.g. Wehr et al. 2017\).](#)

Leaf gas exchange also indicates a large difference in g_1 between C_3 and C_4 species, as expected from their physiology. Although there was a clear difference in $\Delta^{13}C$ between these two groups of species, we were unable to estimate $g_{1\text{-isotope}}$ for the C_4 species and hence unable to substantiate this difference in g_1 at leaf level using isotopic data. The issues involved in estimating C_i/C_a from $\Delta^{13}C$ in C_4 plants are discussed by Cernusak et al. (2013). A simple linear relationship was proposed by Henderson et al. (1992) but requires an estimate of bundle-sheath leakiness, ϕ . Cernusak et al. (2013) suggest that $\phi < 0.37$ under most environmental conditions. With this value of ϕ , the linear relationship yields unrealistic values of C_i/C_a for much of the dataset, as the majority of measured values have $\Delta^{13}C > 4.4\text{‰}$. These data imply that either a value for $\phi > 0.37$ is more commonly found in field conditions, or else that the simple linear relationship between $\Delta^{13}C$ and C_i/C_a is inaccurate for leaf dry matter. Further research is needed to establish more widely-applicable relationships between stable isotope data and water-use efficiency for C_4 species.

Nonetheless, a difference in leaf-level g_1 between C_3 and C_4 species is well-documented in the literature (e.g. Morison & Gifford 1983; Ghannoum et al. 2010). Earlier studies synthesising water-use efficiency from eddy covariance data did not explicitly address photosynthetic pathway (Law et al. 2002, Beer et al. 2009), and thus it was not known whether this fundamental leaf-level difference in g_1 is reflected in canopy scale gas exchange. Zhou et al. (2016) reported a difference in “underlying WUE”, an index similar to g_1 , between C_3 (corn) and C_4 (soybean) crops at 5 Ameriflux sites. Similarly, we found a significant difference in $g_{1\text{-flux}}$ between C_3 and C_4 crops that is consistent with the difference in $g_{1\text{-leaf}}$ (Figure 2). However, we did not find any evidence for lower $g_{1\text{-flux}}$ for grasslands with a C_4 component (Figure 3). The difference in $g_{1\text{-flux}}$ between C_3 and C_4 crops demonstrates that differences in $g_{1\text{-leaf}}$ can scale to whole canopies, and that photosynthetic pathway must be considered when interpreting fluxes from crop canopies. The lack of an influence of photosynthetic pathway on grassland $g_{1\text{-flux}}$, in contrast to

crops, has several potential explanations. It is possible that there are significant evaporative fluxes from soil in grasslands that compensate for differences in transpiration between C3 and C4 vegetation. However, we also note that, owing to a lack of information at site scale, we were obliged to estimate C4 fraction in grasslands from a global dataset with relatively coarse resolution, suggesting that our characterisation of C4 fraction may have been inaccurate. To correctly interpret fluxes from grasslands with a significant C4 component requires better quantification of vegetation C3/C4 fraction at the site level. Furthermore, the estimated grassland C4 fraction did not exceed 0.4; data from grasslands known to have high C4 fraction is needed to test robustly for this effect. Finally, there is very high variability across site-years in g_1 -flux estimates for C3-only grasslands (Figure 3), meaning our test lacks power; a better understanding of the reasons for this variability is needed to design fairer comparisons between C3 and C4-dominated grasslands.

Relative g_1 values from different methods

We predicted that $g_{1\text{-flux}}$ values would exceed $g_{1\text{-leaf}}$ values, due to additional water vapour loss from soil or canopy evaporation (cf. Figure 4). In contrast to our prediction, we found that once decoupling was taken into account, values of median $g_{1\text{-flux}}$ were lower than values of $g_{1\text{-leaf}}$ for several PFTs (Figure 7). Significant within-canopy gradients in $g_{1\text{-leaf}}$ can occur (e.g. Company et al. 2017), but consideration of these gradients would also result in larger $g_{1\text{-flux}}$ than canopy-top $g_{1\text{-leaf}}$. One potential explanation may be related to the use of GPP in the calculation of $g_{1\text{-flux}}$, rather than net photosynthesis (i.e. gross photosynthesis, less leaf respiration) as is used in the calculation of $g_{1\text{-leaf}}$. Recent work by Wehr et al. (2016) also suggests that the current method used to estimate GPP can over-estimate daytime foliar respiration, which would tend to exaggerate the difference between GPP and net canopy photosynthesis. Further research is required to quantify the effect of including foliage respiration in estimation of $g_{1\text{-flux}}$, to determine if this mechanism is sufficient to account for low $g_{1\text{-flux}}$ values.

We also predicted that $g_{1\text{-isotope}}$ values would be ~~somewhat~~ lower than those of $g_{1\text{-leaf}}$ due to mesophyll conductance (g_m), which is neglected in the simplified isotopic theory used

here to relate leaf isotopic composition to C_i/C_a ratio (Seibt et al. 2008). (Evans et al. 1986), though it has been suggested that the value of b used here (Eq. 2) should at least partially account for g_m effects (Seibt et al. 2008, Cernusak et al. 2013). In support of this prediction, median values of $g_{1\text{-isotope}}$ were lower than median values of $g_{1\text{-leaf}}$ for all PFTs other than tropical rainforest (Figure 2). The size of this effect should increase with increasing drawdown of CO_2 from the intercellular airspace to the site of carboxylation; this drawdown is high in plants with low mesophyll conductance (typically ENF and EBF species, Niinemets et al. 2009) and/or high photosynthetic rates. Nonetheless, we were surprised by the magnitude of the difference, which was substantial in most PFTs. Previous smaller-scale studies have found a good correspondence between leaf isotope and gas exchange measurements of C_i/C_a (e.g. Farquhar et al. 1982, Orchard et al. 2010). The size of this difference in our global data comparison suggests that use of the values of $g_{1\text{-isotope}}$ to constrain large-scale models requires that g_m be taken into account. To do so, models will need a general quantitative knowledge of the drawdown of CO_2 from the intercellular space to the mesophyll, which depends on both g_m and the photosynthetic rate (Evans et al. 1986). As woody tissue is generally ^{13}C enriched compared to leaf tissue (Cernusak et al. 2009), values of g_1 estimated from tree ring stable isotopes would likely be lower still.

One exception to this general pattern of lower $g_{1\text{-isotope}}$ values was the TRF PFT (Figure 2). Very high $g_{1\text{-isotope}}$ values were obtained for tropical rainforest species by comparison with other PFTs. These high values may indicate that the leaves used for these measurements were exposed to air with a signature of recent respiration and a correspondingly low ^{13}C fraction, although previous studies suggest that this effect should only be important in the lower canopy (Buchmann et al. 2002). A further potential explanation is that our estimates of long-term average daytime VPD, taken from a global climate dataset (see Methods), do not reflect in-canopy VPD values experienced by sampled leaves, particularly in high-humidity conditions typical of the TRF PFT.

Dataset biases

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Each of the three datasets used in this study represents an enormous global scientific effort, and each is extremely valuable in advancing our understanding of the role of terrestrial vegetation in global carbon and water cycles. Nonetheless, each approach is subject to limitations. Leaf gas exchange measures are a direct and relatively accurate measure of the performance of a single leaf at a given point in time, but are inevitably restricted in sampling coverage. Measurements are often made only at the top of the canopy, for example, or only on a few days per season. There are some more extensive datasets in the Lin et al. (2015) database that were gathered through the use of in-situ cuvettes (e.g. Kolari et al. 2007, Op de Beeck et al. 2010, Tarvainen et al. 2013), but these remain the exception rather than the rule, and in any case cannot capture all potential sources of variation in the canopy. Stable isotope measures are more extensive (Figure 2) but are less direct measures of gas exchange, and, as our results show, may be influenced by other sources of isotopic discrimination. Other potential sources of error in interpreting stable isotope data are the values assumed for long-term average daytime VPD, which are estimated from a global climate dataset (see Methods), and values assumed for source air $\delta^{13}\text{C}$. Eddy flux measurements have the advantage of measuring the behaviour of entire ecosystems, rather than individual leaves. However, these measurements are also subject to noise, and errors may be introduced in the estimation of GPP from measurements of net ecosystem CO_2 exchange (Desai et al. 2008). Furthermore, eddy flux data are known to suffer from an unresolved energy balance problem, in that the sum of latent and sensible heat fluxes is generally less than net radiation (Wilson et al. 2002, Foken 2008). The cause of this imbalance is not yet understood but may differ across sites. There are thus significant uncertainties associated with each of the three datasets. It is also important to be aware of potential bias introduced by different spatial coverage of the three datasets (Figure 1). While we have been able to make some comparisons of different methodologies at specific sites (Figure 6), more such comparisons – and comparisons with isotopic data – would be valuable (e.g. Monson et al. 2010).

With global change accelerating, it is more important now than ever to make use of all available datasets to develop and constrain predictive models of vegetation function.

Cross-comparison of methodologically independent datasets, as we have done here, is a crucial step forward. It highlights areas of inconsistency that should be high priorities for further research. It also quantifies the uncertainty associated with different measurement methods. Finally, our comparison indicates a need for understanding of potential biases when using any or all of these three datasets to constrain or validate ecosystem models that predict WUE.

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Author Contributions

BEM and RAD conceived and designed the study. BEM led writing of paper. MDK and YSL assembled and processed datasets, with assistance from JK, RAD and CAW. AA, RC, PI, J-ML, M-LL, PM, N M-S, and LW assisted with interpretation of datasets. All authors contributed to writing of paper.

Author Information

Data deposition statement: All data and code are available online as follows.

Leaf gas exchange dataset: <https://bitbucket.org/gsglobal/leafgasexchange>

Stable isotope dataset: Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.3jh61>
(Currently under embargo: this address will become live once Cornwell et al. in review paper is accepted)

Eddy covariance dataset: <http://fluxnet.fluxdata.org/data/la-thuile-dataset/>

Analysis code: https://github.com/mdekauwe/g1_leaf_canopy_ecosystem

The authors declare no competing financial interests.

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Table 1: Significant differences among PFTs by Method

Linear mixed models with site as a random factor were applied to gas exchange, isotope and flux data sets separately, and Tukey's HSD used to determine significant differences across PFTs. PFTs with different letters for a given measurement type are significantly different for that measurement type: for example, in the "gas exchange" column, ENF (letter 'a') is significantly different from DBF (letters 'bc') but not EBF (letters 'ac'). Isotope values were log-transformed before analysis. Values of n in table indicate number of sites used for each PFT.

PFT	Gas exchange	n	Isotope	n	Flux	n
ENF (evergreen needleleaf forest)	a	13	a	85	cd	38
EBF (evergreen broadleaf forest)	ac	9	bd	139	bd	7
DBF (deciduous broadleaf forest)	bc	12	bc	108	bc	17
TRF (tropical rainforest)	ab	4	e	95	abd	1
SAV (savanna)	bc	7	de	31	bd	6
SHB (shrub)	ab	6	cd	215	d	4
C3G (C3 grass)	b	2	b	208	d	25
C4G (C4 grass)	a	5	-	-	-	-
C3C (C3 crops)	bc	4	-	-	b	15
C4C (C4 crops)	-	-	-	-	a	7

787 **Table 2: Datasets used for leaf-canopy comparison at individual sites**

788 Details of FLUXNET sites and leaf gas exchange datasets used for leaf-canopy comparison shown in Figure 6.

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FLUXNET Site ID	Lat.	Long.	FLUXNET time period	Gas exchange sampling	FLUXNET reference	Gas exchange reference
AU-Tum	-35.66	148.15	12,1,3 / 2002	Diurnal spot measurements, mid-canopy, 3 campaigns (Nov-01, Feb-02, May-02)	Leuning et al. (2005)	Medlyn et al. (2007)
DK-Sor	55.49	12.10	5,6,7 / 1999	Diurnal spot measurements, upper canopy, 11 dates during Jun-Aug 99	Pilegaard et al. (2003)	Linderson et al. (2012)
FI-Hyy	61.85	24.29	5,6,7 / 2006	Automated shoot cuvette, upper canopy, continuous measurements, Jul-06	Vesala et al. (2005)	Kolari et al. (2007)
FR-LBr	44.72	-0.77	6,7,8 / 1997	Automated branch cuvette, upper canopy, continuous measurements, Sep-97	Berbigier et al. (2001)	Bosc (1999)
Fr-Pue	43.74	3.60	5,6,10 / 2006	First point of A-Ci curves, upper canopy, 11 dates during Apr-Dec 09	Rambal et al. (2003)	Martin-StPaul et al. (2012)
GF-Guy	5.28	-52.93	6,7,8 / 2006	Light-saturated photosynthesis, upper canopy, Oct-10	Bonal et al. (2008)	J. Zaragoza-Castells, O. Atkin, P. Meir, pers. comm.
UK-Gri	56.61	-0.86	5,6,7 / 2001	Automated branch cuvette, upper and mid-canopy, Jul-01	Clement et al. (2003)	Wingate et al. (2007)
US-Ha1	42.54	-72.17	6,7,8 / 1992	Diurnal spot measurements, upper canopy, monthly Jun-Sep 91/92	Urbanski et al. (2007)	Bassow & Bazzaz (1999)

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Figure Captions

Figure 1: Global distribution of datasets used in the study.

Figure 2: Box and whisker plot (line, median; box, inter-quartile range) showing the estimated g_1 values from leaf gas exchange, leaf isotope and FLUXNET data, grouped by plant functional type. Whiskers extend to 1.5 times the inter-quartile range, with dots outside of the whiskers showing outliers. Plant functional types are defined as: ENF - evergreen needleleaf forest, EBF - evergreen broadleaf forest, DBF - deciduous broadleaf forest, TRF - tropical rainforest, SAV - savanna, SHB - shrub, C3G - C3 grass, C4G - C4 grass, C3C - C3 crops, C4C - C4 crops. Values of n indicate no of species for leaf gas exchange and leaf isotope datasets, and no of site-years for FLUXNET. Different letters below boxes denote significant differences among methods for each PFT (Tukey's Honest Significant Difference test, $p < 0.05$). Data shown have been clipped to a maximum g_1 of 14, which excludes 0.0%, 3.18% and 0.22% of leaf gas exchange, leaf isotope and FLUXNET datasets, respectively.

Figure 3: Values of g_{1-flux} for grasslands as a function of the estimated fraction of C4 vegetation.

Figure 4: Values of g_{1-flux} for forest and non-forest vegetation as a function of peak LAI.

Figure 5: Estimated g_1 values from leaf gas exchange, leaf isotope and FLUXNET data, shown as a function of latitude. Where several values were obtained at the same site (different species for leaf gas exchange and isotope, different years for Fluxnet), values have been averaged and standard error bars show variability. Plant functional types are defined as: ENF - evergreen needleleaf forest, EBF - evergreen broadleaf forest, DBF - deciduous broadleaf forest, TRF - tropical rainforest, SAV - savanna, SHB - shrub, C3G - C3 grass, C4G - C4 grass, C3C - C3 crops, C4C - C4 crops. Data shown have been clipped to a maximum g_1 of 14.

Figure 6: Comparison for individual sites between measured leaf-scale stomatal conductance and canopy conductance estimated from FLUXNET as a function of a stomatal index (for gas exchange: $A / (C_a \sqrt{D})$ and for FLUXNET: $GPP / (C_a \sqrt{D})$). Background points show data, darker points show fitted values. Details of gas exchange and FLUXNET measurements are given in Table 2. Measurements were taken from the same year whenever overlapping data were available. The g_1 values shown are the values fitted to the corresponding data.

Figure 7: Box and whisker plot (line, median; box, inter-quartile range) showing the estimated g_1 values from leaf gas exchange, and FLUXNET data calculated using eqn (4) to estimate canopy stomatal conductance (FLUXNET) or the Penman-Monteith equation (eqn 5, FLUXNET-PM). The Fluxnet data are a subset of the data shown in Figure 1 and include only those sites for which eqn 5 could be applied. Whiskers extend to 1.5 times the inter-quartile range, with dots outside of the extent of the whiskers showing outlying values. Plant functional types are defined as: ENF - evergreen needleleaf forest, EBF - evergreen broadleaf forest, DBF - deciduous broadleag forest, TRF - tropical rainforest, SAV - savanna, SHB - shrub, C3G - C3 grass, C4G - C4 grass, C3C - C3 crops, C4C - C4 crops.